Exhibit "C"

The Influence of Buffer Species and Strength on Diltiazem HCl Release from Beads Coated with the Aqueous Cationic Polymer Dispersions, Eudragit RS, RL 30D

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Purpose. Eudragit RL and RS 30D are pseudolatexes frequently used in the coating of solid dosage forms. They are based on cationic copolymers stabilized with quaternary ammonium groups (poly(ethylacrylate-methylmethacrylate-trimethylammonioethyl methacrylate chloride). A pH-independent drug release is expected because of the quaternary nature of the cationic groups. The objective was to explain a distinct "pH-dependent" drug release in various buffer media with coated diltiazem beads.

Methods. The diltiazem HCl release from and water uptake of Eudragit RS/RL-coated beads was determined in various buffers of different buffer species, pH or concentration.

Results. The drug release in the different buffer media was in the following order: pH 5.0 acetate > pH 3.5 formate > pH 7.4 phosphate buffer > 0.1M HCl). This "pH-dependent" drug release could be explained with an anion exchange process; the chloride counterions of the quaternary groups were exchanged with the anionic buffer species during the dissolution study. The water uptake of the coated beads correlated well with the drug release from the beads. Increasing the buffer strength (acetate buffer) first increased and then decreased the drug release, while increasing the ionic strength of different buffers with NaCl decreased the drug release and eliminated the observed buffer effects because of the excess of chloride ions.

Conclusions. The anionic buffer species and not the pH had a significant effect on the hydration and hence on the drug release from beads coated with the cationic polymers, Eudragit RS and RL.

KEY WORDS: beads; buffer species; coating; drug release; latex; Eudragit RS 30D.

INTRODUCTION

Acrylic, water-insoluble polymers have been used extensively to develop oral controlled release drug delivery systems in the form of coated particles, beads or tablets (1-3). These polymers are applied either in the form of organic solutions or as aqueous colloidal dispersions. Eudragit RL and RS 30D are pseudolatexes of poly(ethylacrylate-methylmethacrylate-trimethylammonioethyl methacrylate chloride) copolymers with ratios of 1:2:0.2 and 1:2:0.1 with a polymer content of 30% w/v. The colloidal polymer particles are stabilized in water by the positively charged quaternary ammonium groups present in the polymer. These quaternary ammonium groups are in the chloride salt form. Films or coatings prepared from Eudragit RS or RL 30D are insoluble in aqueous media over the physiological pH-range, however, they swell and hydrate and are permeable to drugs because of the presence of the ionized quaternary ammonium groups.

The permeability of these films and the drug release from coated dosage forms has been described to be pH-independent (4,5). Due to the quaternary groups, the degree of ionization of the polymer should not be affected by pH within the physiological pH range. In this study, surprisingly, a pH-dependent drug release was observed from diltiazem HCl beads coated with Eudragit RS/RL 30D. The solubility of diltiazem HCl was fairly independent of the pH in the investigated pH-range and was not responsible for the observed pH-dependency. The "pH-dependent" release was therefore caused by the polymeric coating.

In a preliminary study, it was shown that the aqueous buffer medium had a significant influence on the hydration and the time-dependent wet mechanical properties of polymeric films prepared from Eudragit RS 30D (6). It was suggested, that the anionic buffer species replaced the chloride-counterions of the quaternary ammonium groups of the polymer during the hydration study and therefore affected the rate and extent of hydration.

The objective of this study was to explain the observed "pH-dependent" drug release from beads coated with the cationic polymers, Eudragit RS/RL. The effect of pH, buffer species, buffer strength and ionic strength on the drug release from the coated beads and the relationship between polymer hydration and drug release were investigated.

MATERIALS AND METHODS

Materials

The following chemicals were obtained from commercial suppliers and used as received: Eudragit RL and RS 30D [poly(ethylacrylate-methylmethacrylate-trimethylammonioethyl methacrylate chloride) copolymers with ratios of 1:2:0.2 and 1:2:0.1] (Röhm, Darmstadt, Germany); acetyl tributyl citrate (ATBC) (Morflex, Inc., Greensboro, North Carolina); sodium phosphate, dibasic; potassium phosphate, monobasic; hydrochloric acid and citric acid, anhydrous (Fisher, Fair Lawn, New Jersey); sodium hydroxide and formic acid, 90% (J.T. Baker, Phillipsburg, NJ); acetic acid, glacial (Mallinckrodt, Paris, Kentucky). Diltiazem HCl powder and diltiazem HCl coated beads were obtained from Marion Merrell Dow (Kansas City, Missouri).

Dissolution and Hydration Media

The following aqueous dissolution media were used: pH 1.0 (0.1 M HCl), pH 3.5 (formic acid-NaOH), pH 5.0 (acetic acid-NaOH) and pH 7.4 (sodium phosphate, dibasic - potassium phosphate, monobasic). The ionic strength of the four

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buffers was kept the same by adding calculated amounts of buffer species (7). The McIlvaines' buffer (citric acid - sodium phosphate, dibasic) was selected to cover a wide pH-range with the same buffer species; the total ionic strength of the buffered solution was adjusted to 0.5 M with NaCl.

Solubility of Diltiazem HCl

Excess amount of diltiazem HCl was placed in the desired buffer medium. The samples were shaken for 48 hours at 37 °C. The saturated drug solutions were filtered and then assayed spectrophotometrically at 236 nm after appropriate dilution (n=3). The final pH of the saturated solution was recorded. The drug solubilities were similar in pH 3.5 formate buffer (652 mg/ml, final pH = 3.46), pH 5.0 acetate buffer (678 mg/ml, final pH = 4.87) and pH 7.4 phosphate buffer (634 mg/ml, final pH = 5.82); the drug solubility in 0.1M HCl was 588 mg/ml. The lower drug solubility in 0.1M HCl solution was probably caused by the common ion effect of the chloride-ion (8).

Dissolution Studies

The USP XXI rotating paddle method (0.2 g beads, 37 °C, 50 rpm, 900 ml medium, n=3, coefficient of variation <5%, dissolution apparatus from Hanson Research Corp., Northridge, California) was used to investigate the drug release from beads coated with Eudragit RS/RL 30D. At predetermined time intervals, samples (2 ml) were withdrawn and replaced with fresh medium. The drug solution was assayed spectrophotometrically either directly or after dilution with the release medium at 236 nm. The residual drug content of the beads after the dissolution study was determined spectrophotometrically after completely crushing the beads with a glass rod. The amount of drug released and the residual drug content in the beads matched the original drug content closely (99.6-104.4%). The release rate and lag time were obtained by a linear regression method from the linear part of the release curve.

Water Uptake of the Coated Beads

At predetermined time intervals, beads (approximately 1 g, accurately weighed) were taken from the release medium with a 60 mesh sieve (the conditions for the water uptake studies were the same as with the dissolution study). The beads were immediately washed twice with distilled water (100 ml) in order to remove the buffer solution from the surface of the beads and were then blotted with lint-free tissue paper. The weight of the beads was recorded before and after drying to constant weight in an oven at 50 °C. The water uptake was calculated as follows: water uptake = W(t) - W (d) / W(d), where W(t) is the weight of the wet beads removed at time t and W(d) is the weight of the beads after drying at time t. The water uptake data are represented as g water / g bead (n=3, coefficient of variation <6%).

RESULTS AND DISCUSSION

In this study, the unexpected "pH-dependent" drug release from diltiazem HCl beads coated with the acrylic polymer dispersions, Eudragit RS and RL 30D, was investigated. Although the drug release has been described to be pH-independent (4, 5), a distinct pH-dependency of the diltiazem HCl release from coated beads was observed (Figure 1). The drug release was determined in media of different pH and of different buffer species (pH 1.0 - hydrochloric acid, pH 3.5 - formic acid-NaOH, pH 5.0 -acetic acid-NaOH and pH 7.4 -sodium phosphate, dibasic-potassium phosphate, monobasic) at a buffer strength of 0.1 M.

The solubility of diltiazem HCl was fairly independent of the pH and could be excluded as the reason for the pH-dependent drug release. The pH-dependent release was therefore caused by the cationic polymeric coating. The drug release profiles had a sigmoidal shape with three phases. A lag phase with little drug release was followed by a rapid, linear release phase followed again by a slow release phase. Especially the extent of the lag time and the rapid release phase were strongly affected by the buffer medium. The drug release was fastest and the lag time shortest with acetate buffer (pH 5.0) followed by formate buffer (pH 3.5), phosphate buffer (pH 7.4) and hydrochloric acid (pH 1.0).

In order to explain the observed "pH-dependent" drug release behaviour, emphasis should be shifted from pH-considerations to the influence of the anionic buffer species present in the dissolution media. An ion exchange mechanism can be used to explain the drug release from the coated beads. Eudragit RS and RL contain 33 and 66 mole of quaternary ammonium groups per mole of polymer (9). The dissociation of these quaternary ammonium groups in aqueous media is responsible for the hydration and swelling of the polymer coating or films. The anionic counterions of the quaternary ammonium groups are chloride ions. With ion exchange resins, ions are bound to an insoluble crosslinked polymer resin carrying oppositely charged functional groups such as quaternary ammonium groups. The affinity of ions to ion exchange resins is characterized by the ion selectivity coefficient. Accordingly, the degree of hydration and swelling of the resins is affected by this interaction (10, 11). Applying this concept to the present study, the chloride counterions of the quaternary ammonium groups in Eudragit RS/RL could be replaced by the buffer anions of the dissolution medium during dissolution studies. The degree of hydration and swelling and subsequently the drug release was governed by the interaction between the cationic groups and the counterions.

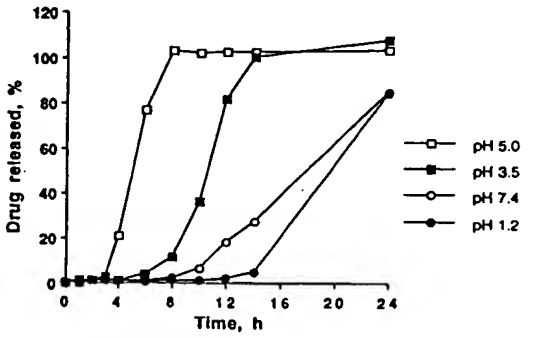

Fig. 1. Effect of different buffers (0.1 M) on the diltiazem HCl release from Eudragit RS/RL 30D coated beads (pH 1.0 - hydrochloric acid; pH 3.5 - formate buffer; pH 5.0 - acetate buffer; pH 7.4 - phosphate buffer).

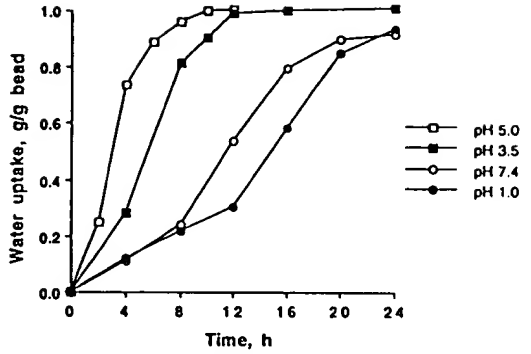

Fig. 2. Effect of different buffers (0.1 M) on the water uptake of Eudragit RS/RL 30D coated beads (pH 1.0 - hydrochloric acid; pH 3.5 - formate buffer; pH 5.0 - acetate buffer; pH 7.4 - phosphate buffer).

The selectivity coefficients of the buffer anions for anion exchangers are in the following order: chloride > formate > acetate (10, 11). A larger selectivity coefficient indicates a stronger interaction between the fixed groups and the counterions and therefore a lesser degree of hydration or swelling; a slower drug release is expected. The order of the selectivity coefficients agreed with the results shown in Figure 1; the order in the drug release (acetate > formate > chloride) was inverse to the order of the selectivity coefficients.

The diffusion of the dissolution medium through and the hydration of the cationic, acrylic polymer coating precedes the drug release through the hydrated polymer film. In order to characterize this hydration phase as a function of pH (buffer species), the water uptake of the beads as a function of time was determined. As shown in Figure 2, the water uptake correlated well with the drug release. The water uptake (swelling) of the Eudragit RS/RL coatings was also in the reverse order of the selectivity coefficients.

The diltiazem HCl release profile and two parameters characterizing the release curve, the release rate and the lag time, as a function of buffer strength (0.01 - 0.5 M, pH 5.0 acetate buffer) are shown in Figures 3 and 4. The drug release rate initially increased with increasing buffer strength and then decreased at higher buffer strength; as expected, the opposite pattern was observed with the lag time. A possible explanation could be as follows. At low buffer strength

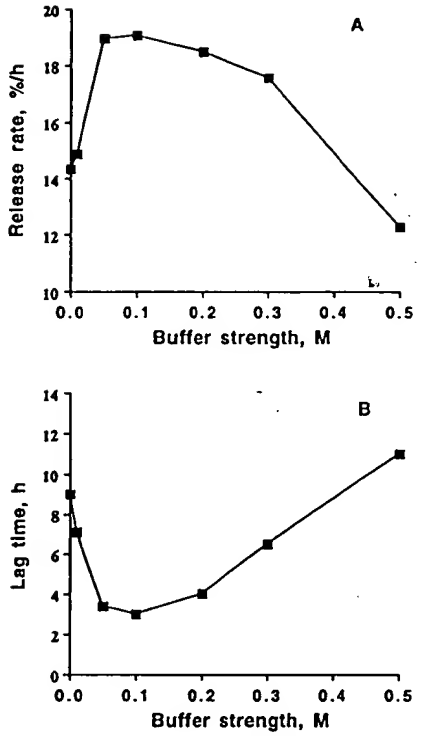

Fig. 4. Effect of buffer strength (pH 5.0 acetate buffer) on the release rate and lag time of the diltiazem HCl release from Eudragit RS/RL 30D coated beads. A: release rate, B: lag time.

(0.01 M), not enough acetate ions were present to replace the original chloride ions. The degree of hydration was therefore governed primarily by the chloride salt form of the polymer, thus explaining the slower drug release. Increasing the acetate concentration resulted in the exchange of the chloride with the acetate ions and therefore in a faster drug release and shorter lag times. The reduction in drug release at high buffer strength could be explained with the high osmotic pressure of the dissolution medium. Again, the water uptake and the rate of water uptake correlated well with the release data (Figures 5 and 6).

Sodium chloride is often added to adjust the ionic

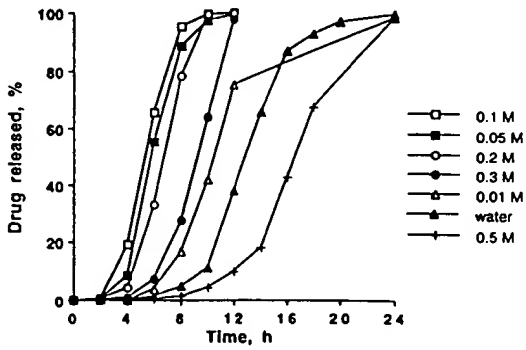

Fig. 3. Effect of buffer strength (pH 5.0 acetate buffer) on the diltiazem HCl release from Eudragit RS/RL 30D coated beads.

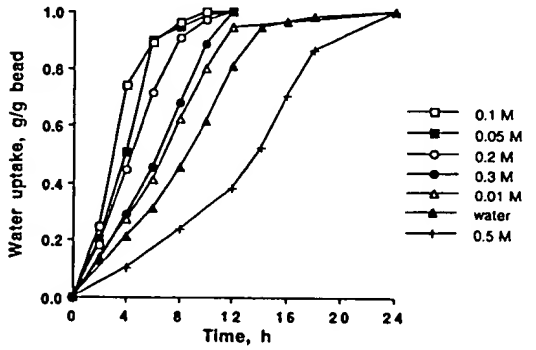

Fig. 5. Effect of buffer strength (pH 5.0 acetate buffer) on the water uptake of diltiazem HCl beads coated with Eudragit RS/RL 30D.

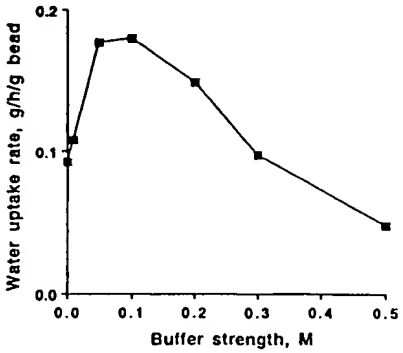

Fig. 6. Effect of buffer strength (pH 5.0 acetate buffer) on the water uptake rate of diltiazem HCl beads coated with Eudragit RS/RL 30D.

strength of different buffer media to the same value. The influence of the addition of NaCl (0.1, 0.4, 0.9 M) to three buffers of the same buffer strength (0.1 M; pH 1.0 - hydrochloric acid, pH 3.5 formate buffer, pH 7.4 - phosphate buffer) was investigated (Figure 7). Without NaCl-addition, the drug release in pH 3.5 buffer was much faster than in pH 1 or pH 7.4 buffers (Figure 7A). However, upon adding NaCl, the differences in the drug release patterns disappeared and the release curves for the three media were almost identical (Figure 7 B-D). The additional chloride counterions in the medium "overpowered" the acetate ions and controlled the hydration and hence the drug release from the beads. As expected the drug release decreased with increasing ionic strength of the dissolution medium. This could be attributed to the lower solubility of the drug (8) and to the higher osmotic pressure of the dissolution medium which decreased the water uptake of the polymer.

The drug release from the coated beads was then tested

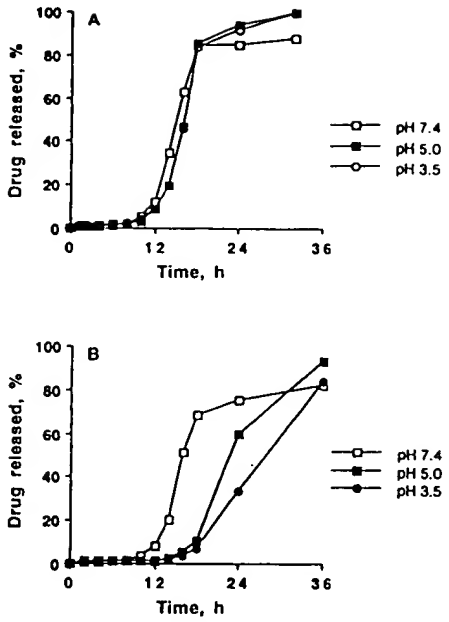

Fig. 8. Effect of pH on the diltiazem HCl release from Eudragit RS/RL 30D coated beads in McIlvaine's buffer. A: at different ionic strength: pH 3.5 - μ=0.13; pH 5.0 - μ=0.25; pH 7.4 - μ=0.46, B: same ionic strength, adjusted to 0.5 M by adding NaCl.

in buffers having different pH values but containing the same buffer species (citrate-phosphate) in varying ratios (McIlvaine's buffer) (Figure 8). The ionic strength of the buffer system at different pH values (pH 3.5, pH 5.0 and pH 7.4), however, was different. The drug release patterns were almost superimposible in buffers having the same type of buffer species but different ionic strengths (0.13 M for pH

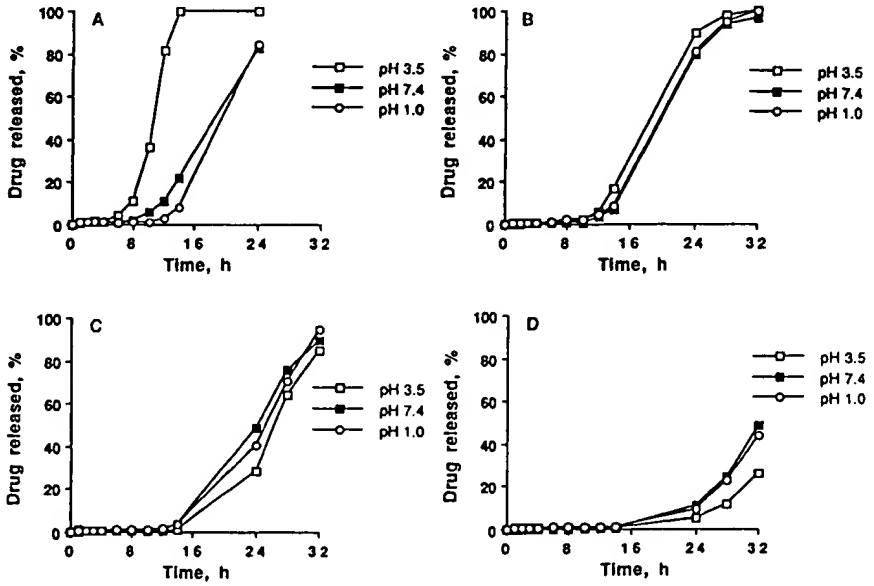

Fig. 7. Effect of NaCl addition to buffers of different pH (0.1M) on the diltiazem HCl release from Eudragit RS/RL 30D coated beads. A: no NaCl, B: 0.1 M NaCl, C: 0.4 M NaCl, D: 0.9 M NaCl.

3.5, 0.25 for pH 5.0 and 0.46 for pH 7.4) (Figure 8 A). In contrast, differences in drug release were observed after adjusting the buffers to the same ionic strength ($\mu = 0.5$ M) with NaCl (26) (Figure 8 B). The drug release was fastest in pH 7.4, followed by pH 5.0 and then pH 3.5, this order being opposite to the amount of NaCl added to adjust to the same ionic strength. As explained above, increasing the amount of NaCl decreased the hydration of the polymeric film and the drug solubility and therefore the drug release.

In conclusion, the anionic buffer species and not the pH had a significant effect on the hydration and hence on the drug release from beads coated with the cationic polymers, Eudragit RS and RL. The buffer-dependent release data could be explained with the ion exchange of the chloride counterions of the polymer with the anionic buffer species during dissolution studies.

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